Stability of Vitamin A in Fortified Wheat Products in Bangladesh

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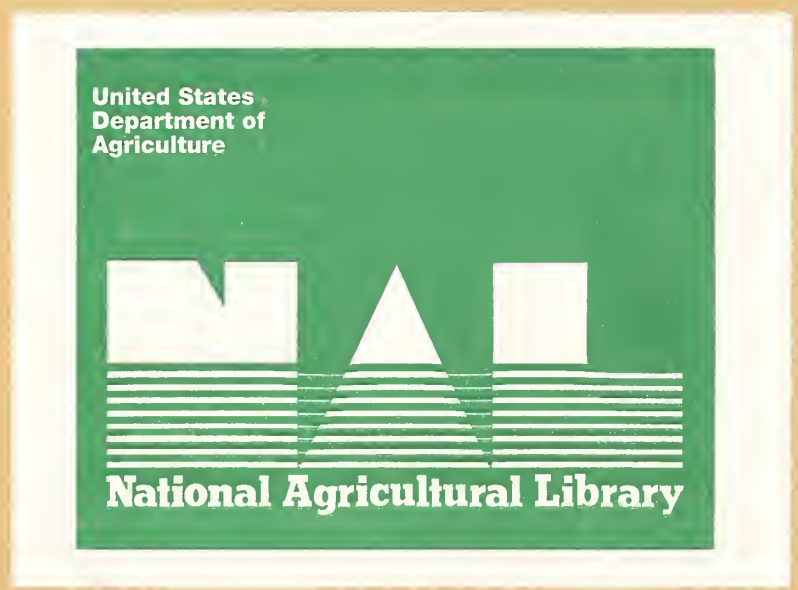
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Project Report

by

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I. Summary

The results of the testing described in this report show that wheat can be fortified to controlled levels of vitamin A by mixing it with a given amount of vitamin A wheat concentratre of known strength.

It was found that when the fortified wheat was ground into whole meal in preparation for use in chapati making or other cooking purposes there was a loss of about 7% of vitamin A potency. This would present no concern regarding nutrient loss.

It is believed that the usual target group recipient in Bangladesh does not have 15 kg of wheat ground at one time in one continuous operation of the chukki mill. Therefore the typical fortified wheat may not be exposed to such a heat load during grinding as was experienced in the conduct of this test in Bangladesh. If this is found to be true, the vitamin A losses found in these tests could be less for the average user of fortified wheat.

There was a 12 percent loss of vitamin A measured during the baking of chapati from fortified whole meal. This level of loss of vitamin A is in-line with the loss of other vitamins when subjected to baking conditions especially in "thinner" products such as crackers, cookies, pancakes, etc.

This loss of vitamin A during the use of vitamin A wheat concentrate may not be of nutritional concern, however, if it is determined that a given level of vitamin A potency is necessary in a chapati when consumed to provide the intended nutritional impact then this loss can be taken into consideration and fortified wheat prepared with approximately 10 percent more vitamin A content to compensate for the processing loss. This approach of adding sufficient vitamin A to compensate for storage and use losses is used commonly in the food industry.

The inter-laboratory comparison indicates that results from Iowa State University and the Institute of Nutrition and Food Science are equivalent and that data gathered in either laboratory would be equally useable for project activities.

The information gained from this testing will be useful in the project for the vitamin A fortification of wheat in Bangladesh and could be helpful in the design and implementaiton of nutrition intervention programs in other countries where vitamin A fortification of whole kernel wheat is a viable option.

II. Background

As part of its program for helping combat vitamin A deficiency and its tragic consequences in Bangladesh, Helen Keller International (HKI), a U.S.-based private voluntary organization, reviewed options for intervention programs for increasing the vitamin A content of the Bangladesh diet. Of particular concern were the participants in the Food for Work (FFW) and Vulnerable Group Development (VGD) programs. With technical assistance from the Food Technology Branch, OICD/USDA, it was concluded that the fortification of imported wheat which is distributed to recipients of these two relief programs could significantly help reduce vitamin A deficiency in 20-25 million people in Bangladesh. These programs are targeted to the poorest and most needy segments of the population and both program can be used to deliver beneficial amounts of vitamin A with relatively small modification in the wheat processing and distribution system of the Government of Bangladesh.

A new process for the addition of high levels of vitamin A to whole wheat kernel to produce a Vitamin A Wheat Concentrate has been developed by the Wright Enrichment Co. in cooperation with the Food Technology Branch. The process involves suspending powdered vitamin A palmitate 250 SD in a mixture of suitable film forming, food grade, edible coating materials including shellac, and ethylcellulose. The suspension is sprayed on the surface of the whole wheat kernels to coat the kernels with vitamin A preparation. Additional layers of coating are applied on top of the vitamin A preparation to help provide chemical stability and to reduce the chance of physical loss of the vitamin A from the wheat during distribution. The vitamin A wheat concentrate is prepared so as to have 2,000,000 International Units (IU) of vitamin A per pound. The wheat concentrate (premix) is designed to be used to make fortified wheat by mixing one part of the premix with 400 parts of regular wheat (0.25% use level) to produce

about 4500 IU of vitamin A per pound of fortified wheat. Concurrent with the in-country technical assistance given regarding silo modification, distribution of fortified wheat and the development of a quality assurance program, testing was carried out to demonstrate the utility of using the vitamin A wheat concentrate to produce fortified wheat in Bangladesh. In addition, studies were done to measure the stability of the vitamin A during typical use by the target families in Bangladesh. Also, testing was done to determine the storage stability of the vitamin wheat concentrate between production and the time the fortified wheat would be ground to meal for consumption. The testing mentioned in this report, unless otherwise indicated was done at the Food Technology Department, Iowa State University as part of the Cooperative Agreement with USDA in support of AID activities on vitamin A fortification of foods in developing countries.

III. Storage Stability of Vitamin A Wheat Concentrate

During development of the technology for producing the whole kernel vitamin A wheat concentrate, tests were conducted to evaluate the storage stability of the product. Accelerated shelf-life testing was done to measure vitamin A loss during storage at several temperature and humidity levels.

A. Objectives. The tests were designed to assess the stability of vitamin A in the whole kernel vitamin A concentrate using accelerated storage testing procedures, to determine the stability of the parent vitamin A preparation used to produce the wheat concentrate kernels, and to collect information on any effects that producing the wheat concentrate kernels might have on extending or reducing the stability of the vitamin A used.

B. Testing. Samples of vitamin A wheat concentrate and of the parent vitamin A palmitate 250-SD used to make the concentrate were tested using an accelerated stability test. The parent material is composed of vitamin A palmitate which has been spray dried (SD) into a matrix containing acacia, lactose, antioxidant, coconut oil, sodium benzoate, and silicone dioxide to produce the product with strength of 250,000 IU of vitamin A per gram of material.The storage conditions used were absence of light, three temperatures, 35°, 45°, and 55°C , and for the wheat concentrate three water activity (humidity) levels (a_w 0.11, 0.75, and 0.90.). The parent vitamin A material was studied only at a_w of 0.11 because at the higher humidities the vitamin A material clumped and gummed-up so that sampling and testing could not be done properly. The procedure for vitamin A assay was to saponify the vitamin A by heating the sample with alcoholic potassium hydroxide then extracting the saponified vitamin A from the mixture with hexane. The vitamin A content of the extract was determined using high performance liquid chromatography (HPLC) procedures.

C. Results. The results of the storage stability tests appear in Table I .

Table I

Stability of Wheat Concentrate

a_w	Temperature	Half Life (weeks) 1/ Parent	Concentrate
0.11	35°C	43	42
	45°	14	15
	55°	6	7

1/ Time required to lose fifty percent of vitamin A potency.

Vitamin losses that occur in storage studies routinely are reported on the basis of a storage temperature of 25°C and ambient humidity which generally is low. In lieu of running tests only at 25°C which takes considerable time, stability data are gathered at higher temperatures and used to prepare a plot of potency losses. With additional calculation this plot can be extrapolated back to a storage temperature of 25°C and the expected loss determined. When the data from Table I are calculated to a typical reporting basis of 25°C and a_w 0.11 the half-life of the wheat concentrate is 56 weeks.

D. Discussion. The results show that the stability of the vitamin A wheat concentrate is quite good and would lose no more than 50 percent potency in one year. They show also that the vitamin A in the wheat concentrate is as stable as the preparation from which it was made. This indicates that there is no loss of vitamin A potency during the processing of the concentrate kernels and that the processing apparently does not change the stability characteristics of the parent material.

The vitamin A palmitate 250-SD material used in these tests is the same as is used for the vitamin A fortification of many foods including the blended foods and commodities used in the PL 480 Title II program.

IV. Vitamin A Stability During Use of Wheat Concentrate

A. Objectives. After having demonstrated the stability of the vitamin A wheat concentrate it was important to gather information on the stability of the vitamin A during grinding, food preparation, and baking it likely would encounter as a part of fortified wheat being used by the target families

in Bangladesh. The conditions of principle concern were (1) the blending of the vitamin A wheat concentrate with regular wheat to make fortified wheat, (2) the grinding of fortified wheat in a Chukki mill to produce meal, and (3) the use of that meal to make chapatis.

B. Testing. Tests were done both in the U.S. at Iowa State University (ISU) and in Dhaka, Bangladesh. Initially, two different tests were carried out in the more controlled conditions of the experimental test kitchen of the Department of Food and Nutrition and the vitamin A assay laboratory of the Department of Food Technology at ISU. These tests were followed by a collaborative field test with the blending, grinding, and chapati baking done in Bangladesh and the vitamin A analyses performed both at the Institute of Nutrition and Food Science (INFS) University of Dhaka and at ISU on the same samples. The field test served both to obtain the needed data from "actual conditions of use" and to give a measure of comparison between the laboratories on procedures and results.

1. Iowa State Test No. 1

Fortified wheat was prepared by blending regular wheat with vitamin A wheat concentrate (Batch No. 14 of development series) at a level of 0.25% to produce a desired level of about 10 IU/g of vitamin A in the fortified wheat The fortified wheat was sampled and assayed for vitamin A in the whole kernel form as was customary when analyzing the concentrate. Then the fortified wheat was ground in a laboratory grinder to a granulation simulating the meal (atta) used in Bangladesh. The meal was used to make chapatis. The meal and chapatis were analyzed for vitamin A content, also. The results appear in Table II.

Table II

Vitamin A Content of Wheat Products Test No. 1

Fortified Wheat	Meal	Chapati
8.15 IU/g ± 2.26	10.49 IU/g ± 1.49	9.77 IU/g ± 0.77
n=3	n=6	n=8

As mentioned earlier, the fortified wheat results are from the extraction of whole kernel samples. This lower than expected mean value for the fortified wheat and the wide range in the standard deviation is attributed to the wide variability in vitamin A content of individual premix kernels. A small difference in the presence of one or two concentrate kernels in a fortified wheat sample can lead to a wide variation in vitamin A results obtained when the assay is done on whole kernels. This is not so apparent when assaying the concentrate when all kernels carry vitamin A.

The vitamin A values in Table II for the different wheat products were analyzed by statistical analysis using Students t test. The results showed these values are not significally different from each other indicating no significant loss of vitamin A occured through the processing steps.

2. Iowa State Test No. 2

A second test was done at ISU utilizing additional information that had been gathered about conditions for grinding and chapati baking. The adjustments more closely simulated the conditions of use in Bangladesh. Again fortified wheat was prepared by blending vitamin A wheat concentrate (Batch No. 14) at the rate of 0.25% with regular wheat to produce a vitamin A level of 10 IU/g. The fortified wheat was ground under more intense degree of grinding in the laboratory grinder to produce a temperature in the meal of about 120°F as it exited the grinder.

This temperature is essentially the same as that measured during the grinding of about 10 kg of wheat in a Chukki mill in Bangladesh. Chapati baking was done as described and demonstrated by a woman from India living at ISU. The dough was made from flour and water at 1.5:1 ratio. It was rested for 15 minutes the divided into 30 g pieces and rounded. Each dough piece was pressed very thin to a diameter of about 5-6 inches. It was baked on an electric grill for about 5 minutes at 1½ minutes on a side with gas bubbles pushed out with a cloth during the last turn. The data on the vitamin A content of the fortified wheat, meal, and chapati appears on Table III.

Table III

Vitamin A Content of Wheat Products Test No. 2

Fortified Wheat	Meal	Chapati
10.0 IU/g	9.64 IU/g ± 0.74	8.28 IU/g ± 0.38
	n=6	n=6

The value shown for fortified wheat is the calculated value based on the vitamin A content of the wheat concentrate used (2,000,000 IU/g) and the amount(0.25%) of concentrate added to regular wheat. Since the fortified wheat value is not the result of vitamin A assay, the apparent loss of

vitamin A during grinding cannot be verified and there can be no statistical treatment of the data for the wheat. However, the meal and chapati data were the results of assay so are suitable for statistical analysis. These data were analyzed using Students t test method and showed a significant difference between the meal and chapati vitamin A values at the 95 percent confidence level. The actual vitamin A values showed a loss of about 14 percent during baking.

The more severe grinding of the wheat accompanied by the increased temperature of the meal seems to have contributed to an increased loss of vitamin A during baking compared to test No. 1. To see if heat build-up alone during the grinding process was contributing to a vitamin A loss, pure vitamin A material and wheat concentrate kernels were heated in an oven at 120°F for from 20 to 30 minutes with samples drawn each two minutes during that time and assayed for vitamin A. There was no measurable reduction in the vitamin A activity of these samples. It is possible that during the grinding step there is a physical disruption of the vitamin A molecule or the matrix material in which it is embedded which makes it more susceptible to destruction due to the heat, moisture, etc. it encounters in the preparation of the chapati.

3. Collaborative Field Test in Bangladesh

The purpose of a collaborative field test was two-fold: First to obtain information of the stability of vitamin A when the wheat concentrate is blended, ground and baked in Bangladesh under conditions it will meet in normal use; and second to obtain a measure of precision from testing the same materials both at ISU and INFS.

The collaborative test was planned by the Food Technology Branch, OICD/USDA and carried out with the cooperation of HKI/Bangladesh, ISU and INFS. A sample of vitamin A wheat concentrate (Batch No. 14) was sent to Bangladesh where it was blended at 0.25% use level with 15 kg of regular wheat to produce fortified wheat. The level of use of the concentrate was selected to produce about 10 IU/g of wheat.

The fortified wheat was ground in a local chukki mill in a continuous feeding run of the entire 15 Kg during which the temperature of the meal reached near 130°F. A sample of the meal was sent to ISU for testing while the remaining meal was used for testing at INFS and for the preparation of chapatis.

The process and equipment used to make the chapatis was the same as that generally used by the poor women in rural areas of Bangladesh. Dough for chapatis was made from flour (meal) and water at 1.8:1 ratio. The dough was divided into pieces 80-85g each and rounded. Each dough ball was rolled very thin to a diameter of 7-7½ inches. A fry pan made from iron sheet was used for the baking. The dough was baked on the pan for 1.0-1.5 minutes at 20-25 seconds on a side with the dough pushed down with a cloth during the last cycle. The temperature of the baked chapati was 90-100°C. When baked the chapatis were sampled in two ways. Fresh (wet) chapatis were sent to ISU for testing and were analyzed immediately by INFS. Dried chapati was prepared by grinding wet chapati in a blender and then drying it in a hot air oven for one hour at 120°F. Samples of dried chapati were sent to ISU and were analyzed by INFS. The ISU samples were sent by DHL express and arrived in seven days.

No sample of fortified wheat was sent to ISU because of the restrictions on bringing raw agricultural material into the U.S. The accuracy of the value for fortified wheat will be interpreted from the precision found in the inter-laboratory comparison.

a. Analysis of Fresh Samples

Samples of fortified wheat, meal and chapatis were assayed for vitamin A content by INFS as soon as they were produced in order to gather information on samples with an age (time after production) as would be found in regular use. The results from the analyses of the fresh samples are in Table IV.

Table IV

Fresh Samples

Sample	Vitamin A (IU.g) 1/ 3/	n	SD 2/	% CV
Premix Concentrate	5003.0	6	331.9	6.63
Fortified wheat	14.29 [a]	4	0.64	4.48
Meal (flour)	13.29 [b]	4	0.44	3.31
Chapati	11.57 [c]	4	0.06	0.52

1/ All data on dry weight basis
2/ Standard Deviation
3/ Values with different letter superscripts are significantly different from each other as analyzed using Students t test ($t_{.05}$)

Vitamin A losses shown are about seven percent during grinding and an additional 12 percent during baking. These losses compare favorably with the results of the ISU Test No. 2 in which the Bangladesh conditions were simulated as nearly as possible.

The results answer the objective of demonstrating the utility of fortifying regular wheat with vitamin A wheat concentrate and showing no excessive vitamin A losses through typical use.

b. Inter-Laboratory Comparison

In addition to the assay of fresh samples, INFS reassayed additional samples of the same material that were held for seven days. This was designed to produce data from samples that would coincide with the assays being performed at ISU on the samples sent air express. Samples in each laboratory would have had comparable age and handling. The purpose of this testing was to gain a measure of the precision of testing the same samples in the two laboratories. Table V shows the data obtained from each laboratory on the same seven day samples.

Table V

Inter-Laboratory Data
for Seven Day Material

	Vitamin A (IU/g) 1/					
	INFS			ISU		
Sample	MEAN	SD	n	MEAN	SD	n
Meal (flour)	12.02	0.50	4	11.12	1.33	6
Chapati (fresh)	11.13	0.49	2	11.93	1.28	5
Chapati (dried)	11.23	0.18	2	10.96	1.13	6

1/ All data on dry weight basis

The inter-laboratory means were statistically analyzed using Students t test and there was found to be no significant differences between the laboratories at the t .05 level. This is valuable to know so that

in the future it could be possible to utilize either laboratory for analysis of samples such as new shipments of wheat concentrate, initial levels of vitamin A in fortified wheat, etc. in either country with meaningful and useful results.